Cellular ROS and Lipid ROS detection

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Detailed protocol

For cellular ROS staining:
1. Grow cells: on the day of the assay, centrifuge cells from the culture medium and resuspend the cell pellet in culture medium at 5 x 10^4 – 10^5 cells/90 µL per cell in coated 96-well plate.
2. Prepare CM-H2DCFDA or Deep Red ROS working solution (Add 20 µL of stock solution to 10 mL of Assay Buffer and mix well, final working solution: 5-50 µM).
3.(optional) Treat cells with 10 µL of 10X test compounds in PBS or HHBS.
4. Add 100 µL/well of CM-H2DCFDA or Deep Red ROS Working Solution into the cell plate.
5. Incubate cells in a 37ºC/5% CO2 incubator for 30 – 60 minutes.
6. Monitor fluorescence intensity at Ex/Em =488/30 or 650/675 nm (cut off = 665 nm) by flow cytometry.

For lipid ROS (using lipid peroxidation kit)
1. Seed cells at desired density in 24-well (1-5 million) or 96-well (0.04-0.2 million) plate .
2. (optional) Treat cells with test compounds as desired. (For a positive control, add hydrogen peroxide to the cells at a final concentration of ~250 µM (1X) for 30 minutes)
3. Add Lipid Peroxidation Sensor stock solution to cell medium at a final concentration of 50-100 µM.
4. Incubate the cells for 30 min at 37°C with 5% CO_2 cell incubator.
5. Remove media and wash cells with HHBS or DPBS three times.
6. Monitor fluorescence of cells with flow cytometer through FITC/TRITC or FITC/PE channels within 2 hours of staining.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Xiao, L. (2022). Cellular ROS and Lipid ROS detection. Bio-protocol Preprint. bio-protocol.org/prep1763.

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